Guidance

S9.73 - Elaboration of limit values for Polycyclic Aromatic Hydrocarbons in oil for feed based on the PAH4 methodology

Version EN: 1 March 2021

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TNO report

TNO2012 R10605 | 1

Elaboration of limit values for Polycyclic Aromatic Hydrocarbons in oil for feed based on the PAH4 methodology

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Copy no	-
No. of copies	4
Number of pages	9 (incl. appendices)
Number of appendices	-
Sponsor	GMP+ International Braillelaan 9 2289 CL Rijswijk ZH Nederland
Project number	051.02499/01.01

1 Introduction

GMP+ International establishes limit values for contaminants in feed. Within this framework, GMP+ International is currently reassessing its limit values for polycyclic aromatic hydrocarbons (PAHs) in feed. Currently, GMP+ International uses the PAH12 norm for the measurement of polycyclic aromatic hydrocarbons in feed products. This norm is based on the sum of acenaphtene, acenaphtylene, benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, indeno(1,2,3-cd)pyrene, phenanthrene and pyrene as suitable indicators of PAH in feed. The norm is introduced in the past to prevent PAHs in feed as a result of contamination by mineral oils.

In 2008, the EFSA published a scientific opinion[1] in which it advises the measurement of PAH4 in food products. The PAH4 constitutes the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene in food products. In 2011 the Commission Regulation (EU) No 835/2011 amending Regulation (EC) 1881/2006 has adopted the proposed method by the EFSA. This regulation shall apply from 1 September 2012.

GMP+ International has requested TNO to advise on the norm setting for PAH4 in fat for feed. For this purpose, GMP+ international has supplied analytical data concerning measurements of PAHs in the concerned fat for feed products from three different companies. Moreover, GMP+ International requested to evaluate the outcome of the analytical data provided comparing the amount of data exceeding the proposed norm setting for PAH4 compared to the PAH12 norm.

The dataset containing the analytical data from PAH measurements in fat for feed products is composed, among others, by the following products: crude palm oil, mixed vegetable acid oil, soya lecithin, and coconut fatty acid distillate.

It should be noted that in Commission Regulation (EU) No 835/2011, as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs, the following was stated in consideration no. 19: '*coconut oil can contain higher amounts of PAH4 than other vegetable oils. This is due to the proportionally higher presence of benz(a)anthracene and chrysene which cannot be easily removed during refinement of coconut oil. Specific maximum levels for coconut oil should therefore be set at levels as low as reasonably achievable and taking into account the current technological possibilities of producing countries. As technological improvements in producing countries are expected, the levels of PAH in coconut oil should be regularly monitored with a view to assessing the possibility for setting lower levels in future.*'. Therefore, it was decided to calculate separate norm settings for coconut/palm oil and other vegetable oils.

[1] EFSA (2008) Polycyclic Aromatic Hydrocarbons in food. Scientific opinion of the panel on contaminants in the food chain. *The EFSA Journal:* 724: 1-114

2 Methodology

2.1 Datasets provided

GMP+ international provided 3 Excel sheets with reportedly fat for feed products provided by 3 different companies as confidential information. The total dataset used consists of 546 analytical samples of PAH12 measurements covering April to December 2011, January to December 2011, and January 2010 to March 2012 for the respective companies. It should be noted that a limited amount of samples were available which did not comply with the current PAH12 norm (sum of PAH12 exceeded the rejection norm of 50 µg/kg BaPEQ)

The datasets provided were converted to the same format and merged thereafter. All analytical results indicated as 'less than' were used in the calculations at the respective limit of quantification (LOQ) value.

In one of the datasets for a few samples identical copies of data were located. Double datasets were deleted. Furthermore, for some samples identical analytical data were provided for the same sample number but with a different sample description (e.g. Coconut fatty acid distillate and Palm fatty acid distillate). Also for these data one of the set of PAH data was deleted.

In a second dataset, some PAH levels were indicated as NM (not measured). These data were omitted from the calculations. Furthermore, some blank cells were found throughout the dataset, which were also present within the PAH4 substances. These data were assumed to be below the respective LOQ. As the LOQ was not provided, the lowest value present in this dataset for the respective PAH was taken as the LOQ value for the blank cells. A single set of data showed all PAH levels at 0. As it is unlikely that all PAHs measured in a sample are 0 and an LOQ of 0 is not possible, this sample was omitted from the calculations.

In a third dataset, some blank cells were found throughout the dataset. These data were assumed to be below the respective LOQ. The LOQ values from other samples were taken as the LOQ value for these blank cells.

2.2 Calculating PAH4 norm

To calculate the norm for PAH4 as the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene, the methodology for calculating maximum residue levels (MRLs) for pesticides was applied as described in the guidelines for evaluation of residues according Directive 91/414/EEC, Commission of the European Communities, document 7039/VI/95: Appendix I - Calculation of Maximum Residue Levels and Safety Intervals e.g. Pre-harvest Intervals. In this guideline, two methods for the calculation of maximum residue levels are described. The Method I assumes a normal distribution for the measured residues, taking into account arithmetic mean, standard deviation and maximum residues per sampling time. This method assumes a number of residue figures between 0 and 10. The Method II takes into account that a normal distribution cannot always be assumed with sufficient certainty for results from residue tests. The method uses a

distribution-free parameter (quantile) and is designed the produce maximum residue levels in the normal order of magnitude given the amount of data material which is usually available.
For the present evaluation the Method II was applied given the fact that the data supplied by the three companies includes an amount of 546 samples, for which Method I is not suitable for the present evaluation.

This method was applied to both the sum of benzo[a]pyrene equivalent corrected PAHs for the PAH12 and the sum of PAH4 results. The PAH12 calculation was used as verification of the dataset with respect to the current norm (action limit of 15 µg/kg BaPEQ, and rejection norm of 50 µg/kg BaPEQ), to determine if the dataset used is representative based on the current PAH12 norm.

Calculation method
Median and quantiles are the obvious distribution-free parameters for the metric data available. With the few test series available, a quantile greater than the 75 % one is not practicable. The 75 % quantile is to be preferred to the median because it gives a more accurate representation of the frequently negative skew of the distribution function. Lastly, the maximum residue levels should primarily take into account the maximum values possible and not reflect the central distribution trend.
This calculation has the advantage over other quantile calculations in that a weighted average is determined at point R (P · (n+1)) taking into account the figures recorded empirically. The method is relatively insensitive to a variation in n.

The following equations are used to calculate the maximum limit for both PAH12 and PAH4:

R(0.75) = (1 - G) · R(J) + G · R(J+1)
where (n+1) · P = J + G

n = number of values
P = T/100, in this case 0.75
T = percentile, in this case 75 %
J = whole-integer proportion of (n+1) · P
G = fraction of (n+1) · P
R (J) = residue value at point J
R (J+1) = residue value at point J + 1
R (0.75) = desired quantile

Calculation of the proposed maximum level:

R(ber) = 2 · R(0.75)
R (ber) = calculated figure for the proposed maximum level

3 Results

For each of the samples in the dataset the PAH12 was calculated as the sum of benzo[a]pyrene equivalent (BaPEQ) corrected values. Furthermore, the sum of PAH4 was calculated separately for coconut/palm oil and other vegetable oils. Thereafter the statistical calculations were performed according to paragraph 2.2.

The derived values for the calculations of the respective PAH12 and PAH4 is given in Table 1.

Table 1 – Calculation of the maximum residue level according to the PAH12 and PAH4 norm

	Sum of PAH12 (µg/kg BaPEQ)	**Coconut/palm oil, sum of PAH4** (µg/kg)	**Other vegetable oils, sum of PAH4** (µg/kg)
Mean	16.9	103	59.2
SD	26.4	117	93.4
min	0.67	1.3	1.3
max	290.4	677.5	840.7
N	546	211	335
J	410	159	252
G	0.25	0	0
R(J)	21.76	177.0	69.5
R(J+1)	21.84	177.2	69.7
R(0.75)	21.78	177	69.5
R(ber)	43.56	354	139

For the PAH12 figures, the mean value of 16.9 µg/kg BaPEQ is close to the current action limit of 15 µg/kg BaPEQ, whereas the calculated maximum limit R(ber) of 43.56 µg/kg BaPEQ , assigned to the next higher value of 10 or the next higher class, is 50 µg/kg BaPEQ, being the current rejection norm for PAH12. These figures are confirming the validity of the dataset to be used for the calculations of norm settings.

3.1 PAH4 rejection norm setting

Based on the above, the calculated maximum levels R(ber) for coconut/palm oil and other vegetable oils can be assigned to a PAH4 rejection norm for coconut/palm oil of 400 µg/kg and for other vegetable oils of 200 µg/kg, considering the next higher value of 100.

Within the coconut/palm oil dataset used, 12 samples (5.7%) were not conform the PAH12 norm whereas 6 samples (2.8%) were exceeding the proposed norm of 400 µg/kg PAH4 out of 211 samples. From the samples exceeding the proposed PAH4 norm, 5 out of 6 samples were also rejected based on the PAH12 norm.

Within the other vegetable oil dataset used, 5 samples (1.5%) were not conform the PAH12 norm whereas 20 samples (6.0%) were exceeding the proposed norm of

200 µg/kg PAH4 out of 335 samples. All the samples exceeding the proposed PAH4 norm were also rejected based on the PAH12 norm.

Although a significant amount of vegetable oil samples are exceeding the proposed PAH4 norm, related to the amount of samples exceeding the PAH12 norm, it should be noted that for the total dataset the difference is less clear. A total of 17 samples are to be rejected based on the PAH12 norm whereas this is 26 for the proposed PAH4 norm, which is not considered a significant difference taken into account a sample size of 546. In case the proposed norm for PAH4 in other vegetable oils would be raised to 300 µg/kg, the amount of rejected samples is 7, of which 5 were also rejected based on the PAH12 norm. From a precautionary point of view, considering the carcinogenic potential of PAHs, a rejection norm of 200 is preferred over a rejection norm of 300 µg/kg. Furthermore it is noted that for foodstuffs, maximum levels for PAH4 in coconut oils and in Oils and fats (excluding cocoa butter and coconut oil) are set by Commission Regulation (EU) no 835/2011 at a level of 20 and 10 µg/kg, respectively. These levels are a factor 2 apart, confirming the choice of a PAH4 norm setting of 400 and 200 µg/kg for coconut/palm oils and other vegetable oils in fats for feed, respectively.

3.2 PAH4 action limit settings

GMP+ International decided to set action limits at 80% of the concurrent rejection norms. Based on the rejection norms elaborated in paragraph 3.1, PAH4 action limits of 320 µg/kg for coconut/palm oil, and 160 µg/kg for other vegetable oils, are proposed.

3.3 Analysis

PAHs can be analysed by using GC-MS or HPLC-FLD as an analytical method. For the analysis in food, analytical methods are provided by the EFSA in 2008 (see paragraph 3.2[2]), whereas analytical specifications are laid down in EU Commission Regulation 333/2007[3]. For feed, analytical methods are reported by the RIKILT in 2001[4]. These analytical techniques concern the detection of benzo(a)pyrene equivalents, but also measure the PAH4 set of markers, being the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene. The analytical method of use should comply with the general analytical criteria as set for, amongst others, the Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, specificity, precision and recovery.

[2] EFSA (2008) Polycyclic Aromatic Hydrocarbons in food. Scientific opinion of the panel on contaminants in the food chain. *The EFSA Journal:* 724: 1-114

[3] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:088:0029:0038:EN:PDF

[4] Rikilt 2001, W. Traag, Polycyclische aromatische koolwaterstoffen (PAK's) in diervoededers, dierlijke vetten, plantaardige oliën/vetten, vetzuren en dergelijke. Report no. 3001.006: http://www.rikilt.wur.nl/NR/rdonlyres/BDEEDD31-F58C-47EB-A0AA-23CB9956CE18/128454/R2001006NED.pdf (date 18.09.2012)

4 Conclusion

GMP+ International has requested TNO to recommend a norm setting for polycyclic aromatic hydrocarbons in fats for feed based on the PAH4 methodology as proposed by the EFSA.

Based on analytical data from three different companies, maximum levels were calculated using the methodology to set pesticide residue MRLs. For verification purposes also calculations were performed for the PAH12 methodology, for which the current norm is set.

The validity of the dataset is confirmed as the mean value of the PAH12 calculation was near the action limit and the calculated maximum level was equal to the current norm of 50 µg/kg BaPEQ.

For oils and fats (excluding palm(kernel)- and coconut oil, and products derived thereof) an action limit of 160 µg/kg and a rejection limit of 200 µg/kg is proposed for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene (PAH4).

For palm(kernel)- and coconut oil, and products derived thereof, an action limit of 320 µg/kg and a rejection limit of 400 µg/kg is proposed for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene (PAH4).

It should be noted that the PAH4 methodology is based on the sum of the respective PAHs without conversion, which is different than the current PAH12 methodology for which the analytical data are corrected for their respective toxicological equivalence factors.

The proposed PAH4 rejection norms for oil for feed were evaluated by TNO with respect to the transfer of the concerned PAH's to edible animal commodities[5]. It was concluded that it is unlikely that the proposed norms for oil for feed, taken into account the worst case assumptions used, will lead to levels at or above the authorized levels of PAH4 in smoked meat or smoked meat products, not taken into account the contribution of PAH4 as a result of smoking.

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[5] TNO report TNO2012 R10606

5 Signature

Zeist, September 2012

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At GMP+ International, we believe everybody, no matter who they are or where they live, should have access to safe food.

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